

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference N.75121A PEJ	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB99/03873	International filing date (day/month/year) 19/11/1999	Priority date (day/month/year) 19/11/1998
International Patent Classification (IPC) or national classification and IPC C12N15/53		
Applicant ISIS INNOVATION LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 8 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 15/06/2000	Date of completion of this report 27.02.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Strobel, A Telephone No. +49 89 2399 7362 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03873

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*

Description, pages:

1-45 as originally filed

Claims, No.:

1-20 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-16, filed with the letter of 8.2.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03873

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 15-17 completely, 1-14, 18-20 all partially.

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
 - ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 - ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 - ☒ no international search report has been established for the said claims Nos. 15-17 completely, 1-14, 18-20 all partially.
2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
 - ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03873

1. Statement

Novelty (N)	Yes:	Claims	5-7, 9-14, 18-20
	No:	Claims	1-4, 8:
Inventive step (IS)	Yes:	Claims	1-4, 8
	No:	Claims	5-7, 9-14, 18-20
Industrial applicability (IA)	Yes:	Claims	1-14, 18, 20
	No:	Claims	

2. Citations and explanations see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/03873

Re Item I

Basis of the opinion

The claimed priority has not been checked since the International Search Report does not cite documents published after the priority date.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

In the International Search Report, an objection of lack of unity was raised. Since the applicant neither restricted the claims nor paid additional fees, the International Search Report was only established for the invention of group one of claims 1-14, 17-20, all partially. Claims or part of claims for which no International Search Report has been established need not be the subject of an International Preliminary Examination (Rule 66.1(e)PCT). In consequence, the Preliminary Examination Report covers only the process of oxidising a terpene or cycloalkene by using a mutant P450cam in which amino acid 96 has been changed to an amino acid with a less polar side chain, also a nucleotide encoding it and a cell expressing it. Since claim 17 depends on claims 15 and 16, said claim is also not subject of the present Written Opinion.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: GB 2 294 692 A (BRITISH GAS PLC) 8 May 1996 (1996-05-08)
- D2: ATKINS W M ET AL: "Molecular recognition in cytochrome P-450: alteration of regioselective alkane hydroxylation via protein engineering." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 111, 1989, pages 2715-2717, XP002131692 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863
- D3: ENGLAND P A: "The oxidation of naphtalene and pyrene by cytochrome P450cam" FEBS LETTERS., vol. 424, 13 March 1998 (1998-03-13), pages 271-274, XP002131695 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0014-5793

D4: ATKINS W M ET AL: "THE ROLES OF ACTIVE SITE HYDROGEN BONDING IN CYTOCHROME P-450 CAM AS REVEALED BY SITE-DIRECTED MUTAGENESIS" JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 263, no. 35, 15 December 1988 (1988-12-15), pages 18842-18849, XP002025958 ISSN: 0021-9258

1. This application concerns a process of oxidising terpenes or cycloalkenes by means of a mutant P450cam haem-containing monooxygenase in which amino acid residue 96 has been replaced by an amino acid with a less polar side chain. Applicants claim said process, a process wherein said P450cam mutant contains additional substitution mutations, a polynucleotide encoding such a mutant P450cam enzyme, cells expressing these mutants alone or with electron transfer reductases and electron transfer redoxins. Further claimed is a said process of oxidising terpenes or cycloalkanes within a cell that expresses the mutant P450cam enzyme.

2. Novelty of claims 1-4 and 8

D1 describes a process of oxidising substrates by means of a mutant P450cam enzyme in which the natural amino acid residue at position 96 (tyrosine) is replaced by a residue with a less polar side chain, e.g. alanine. D1 also discloses further mutants of P450cam with point mutations at at least one of positions 87, 98, 185, 244, 247, 295, and 297 (abstract, p. 3, first and third paragraph, p.5, third paragraph, in vitro oxidation protocol: p.10, last paragraph-p.13). Using the process described in D1, the mutant P450cam Y96A is capable of oxidising cycloalkenes (table 2(a)) and terpenes (table 2(g), e.g. isoprene; and table 2(h), e.g. fenchone).

D2 describes a process for in vitro oxidation of the bicyclic terpenes, camphor, norcamphor, CH₃-norcamphor, and a halogenated camphor derivative, by using the mutant P450cam Y96F (figure 2 and legend, p. 1715, right column, p. 2716, right column, third paragraph).

D3 discloses several P450cam mutants carrying substitutions at position 96 (Y96G, Y96A, Y96V, Y96F), which are used in an in vitro process of oxidising camphor (table 1, Materials and Methods section).

D4 describes the construction of a P450cam mutant Y96F by site-directed

mutagenesis, the expression of said mutant in E.coli, its purification and an in vitro process for the oxidation of camphor and synthetic analogues (abstract; Materials and Methods, point 2.3; p. 18843, right column third paragraph; p.18845, right column, second paragraph; table 3).

Thus, each of D1, D2, D3, and D4 on its own destroys novelty of claims 1, 2, and 3.

D1 furthermore takes away the novelty of claim 4. D4, which describes E.coli cells expressing the mutant P450cam Y96F, destroys the novelty of claim 8, since any E.coli cell contains an electron transfer reductase and an electron transfer redoxin.

Thus, claims 1, 2, 3, 4, and 8 do not fulfil the requirements of Article 33(2)PCT.

3. Inventive step of claims 5-7, 9-14, 18-20

The additional features of the present claims 5-7, 9-14, 18-20 are either trivial or conventional in the art or within the competence of a skilled man seeking to improve the prior art processes mentioned in the search report and in the present opinion, so that the subject-matter of said claims also lacks an inventive step (Article 33(3) PCT).

4. Disclaimers in claims 6-11

An additional indication of the obviousness of the claims dealt with under V.3 are the disclaimers defined in claims 6 and 9 (and consequently in claims 7, 8, 10, and 11). Claim 6 avoids thereby D1, and claim 9 excludes thereby the otherwise novelty destroying prior art document D3. However, D1 and D3 belong to the very same technical field as the application, namely the oxidation of compounds by means of mutant P450cam enzymes. A disclaimer is intended to give the opportunity to applicants to avoid prior art that incidentally destroys novelty of a claim. Disclaimers are not intended to establish inventiveness. This means that said disclaimers cannot render claims 6-11 non-obvious with respect to D1 and D3. If this were the case, any claim could be made inventive simply by introducing disclaimers that would exclude the prior art, be it as relevant and close as possible.

5. Industrial applicability of claim 19

For the assessment of the present claim 19 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

1. Claim 1 refers to a process for oxidising a terpene or cycloalkene, which process comprises oxidising said compound with a **mutant haem-containing enzyme** (emphasis added). However, haem-containing enzymes constitute a vast class of different proteins, displaying numerous different functions (cytochromes for instance in the respiratory chain, oxidoreductases etc.). The application concerns only a specific cytochrome P450, namely a mutant P450cam, or species homologues thereof. Thus, claim 1 is not supported by the description, since it is not shown in the application that haem-containing enzymes other than a mutant P450cam is capable of performing said process (Non-fulfilment of Article 6 PCT).
2. The disclaimer of dependent claim 6, which refers to claims 4 or 5, is not comprised in claim 5. Thus, the subject-matter of claim 6 is not defined clearly enough (Article 6 PCT).
3. Claims 9-12 concern cells comprising a mutant P450cam enzyme or a fragment thereof. It is not clear what these fragment could be. Said claims do not satisfy the requirements of Article 6 PCT.

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 16 August 2000 (16.08.00)	
International application No. PCT/GB99/03873	Applicant's or agent's file reference N.75121A PEJ
International filing date (day/month/year) 19 November 1999 (19.11.99)	Priority date (day/month/year) 19 November 1998 (19.11.98)
Applicant WONG, Luet, Lok et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

15 June 2000 (15.06.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Pascal Piriou Telephone No.: (41-22) 338.83.38
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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) N.75121A PEJ

Box No. I TITLE OF INVENTION

PROCESS FOR OXIDISING TERPENES

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

ISIS INNOVATION LIMITED
2 South Parks Road
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United Kingdom

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (that is, country) of nationality:
GB

State (that is, country) of residence:
GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

WONG, Luet Lok
University of Oxford
Department of Chemistry
South Parks Road
Oxford OX1 3QR
United Kingdom

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
GB

State (that is, country) of residence:
GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent ☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

ELLIS-JONES, Patrick George Armine
J.A. KEMP & CO.,
14 South Square,
Gray's Inn,
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+44 171 405 3292

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23676

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS	
<i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p> <p>BELL, Stephen Graham University of Oxford Department of Chemistry South Parks Road Oxford OX1 3QR United Kingdom</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
<p>State (that is, country) of nationality: GB</p>	<p>State (that is, country) of residence: GB</p>
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p> <p>CARMICHAEL, Angus Bishop University of Oxford Department of Chemistry South Parks Road Oxford OX1 3QR United Kingdom</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
<p>State (that is, country) of nationality: GB</p>	<p>State (that is, country) of residence: GB</p>
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
<p>State (that is, country) of nationality:</p>	<p>State (that is, country) of residence:</p>
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
<p>State (that is, country) of nationality:</p>	<p>State (that is, country) of residence:</p>
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p><input type="checkbox"/> Further applicants and/or (further) inventors are indicated on another continuation sheet.</p>	

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked).

Regional Patent

- ☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | |
|---|---|
| <input checked="" type="checkbox"/> AE United Arab Emirates | <input checked="" type="checkbox"/> LR Liberia |
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> LS Lesotho |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> LT Lithuania |
| <input checked="" type="checkbox"/> AT Austria | <input checked="" type="checkbox"/> LU Luxembourg |
| <input checked="" type="checkbox"/> AU Australia | <input checked="" type="checkbox"/> LV Latvia |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> MD Republic of Moldova |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina | <input checked="" type="checkbox"/> MG Madagascar |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> MA Morocco |
| <input checked="" type="checkbox"/> BR Brazil | <input checked="" type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> MW Malawi |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> MX Mexico |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein | <input checked="" type="checkbox"/> NO Norway |
| <input checked="" type="checkbox"/> CN China | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> CU Cuba | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> CZ Czech Republic | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> DE Germany | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> DK Denmark | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> EE Estonia | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> FI Finland | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> GB United Kingdom | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> GD Grenada | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> GE Georgia | <input checked="" type="checkbox"/> SL Sierra Leone |
| <input checked="" type="checkbox"/> GH Ghana | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> GM Gambia | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> HR Croatia | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> HU Hungary | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> ID Indonesia | <input checked="" type="checkbox"/> UA Ukraine |
| <input checked="" type="checkbox"/> IL Israel | <input checked="" type="checkbox"/> UG Uganda |
| <input checked="" type="checkbox"/> IN India | <input checked="" type="checkbox"/> US United States of America |
| <input checked="" type="checkbox"/> IS Iceland | <input checked="" type="checkbox"/> TZ Tanzania |
| <input checked="" type="checkbox"/> JP Japan | <input checked="" type="checkbox"/> UZ Uzbekistan |
| <input checked="" type="checkbox"/> KE Kenya | <input checked="" type="checkbox"/> VN Viet Nam |
| <input checked="" type="checkbox"/> KG Kyrgyzstan | <input checked="" type="checkbox"/> YU Yugoslavia |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> ZA South Africa |
| <input checked="" type="checkbox"/> KR Republic of Korea | <input checked="" type="checkbox"/> ZW Zimbabwe |
| <input checked="" type="checkbox"/> KZ Kazakhstan | |
| <input checked="" type="checkbox"/> LC Saint Lucia | |
| <input checked="" type="checkbox"/> LK Sri Lanka | |

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☒ **CR** Costa Rica
- ☒ **DM** Dominica

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM		<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.		
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application:* regional Office	international application: receiving Office
item (1) 19 NOVEMBER 1998	9825421.2	GB		
item (2)				
item (3)				
<input checked="" type="checkbox"/> The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): (1)				
<small>* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.</small>				
Box No. VII INTERNATIONAL SEARCHING AUTHORITY				
Choice of International Searching Authority (ISA) <small>(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):</small>		Request to use results of earlier search; reference to that search <small>(if an earlier search has been carried out by or requested from the International Searching Authority):</small>		
ISA /		Date (day/month/year)	Number	Country (or regional Office)
Box No. VIII CHECK LIST; LANGUAGE OF FILING				
This international application contains the following number of sheets: request : 4 description (excluding sequence listing part) : 53 claims : 4 abstract : 1 drawings : 4 sequence listing part of description : _____ Total number of sheets : 66		This international application is accompanied by the item(s) marked below: 1. <input checked="" type="checkbox"/> fee calculation sheet 2. <input type="checkbox"/> separate signed power of attorney 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: 4. <input type="checkbox"/> statement explaining lack of signature 5. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s): 6. <input type="checkbox"/> translation of international application into (language): 7. <input type="checkbox"/> separate indications concerning deposited microorganism or other biological material 8. <input type="checkbox"/> nucleotide and/or amino acid sequence listing in computer readable form 9. <input checked="" type="checkbox"/> other (specify): Form PF 23/77		
Figure of the drawings which should accompany the abstract:		Language of filing of the international application: ENGLISH		
Box No. IX SIGNATURE OF APPLICANT OR AGENT				
Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request). ELLIS-JONES, Patrick George Armine				

For receiving Office use only	
1. Date of actual receipt of the purported international application: _____ 3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: _____ 4. Date of timely receipt of the required corrections under PCT Article 11(2): _____ 5. International Searching Authority (if two or more are competent): ISA /	2. Drawings: <input type="checkbox"/> received: <input type="checkbox"/> not received: 6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.

For International Bureau use only
Date of receipt of the record copy by the International Bureau: _____

The demand must be filed directly with the competent International Preliminary Examining Authority or, if there are more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only	
Identification of IPEA	Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION	
Applicant's or agent's file reference N.75121A PEJ	
International application No. PCT/GB99/03873	International filing date (day/month/year) 19 November 1999 (19/11/99)
(Earliest) Priority date (day/month/year) 19 November 1998 (19/11/98)	
Title of invention PROCESS FOR OXIDISING TERPENES	
Box No. II APPLICANT(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
ISIS INNOVATION LIMITED Ewert House Ewert Place Summertown Oxford OX2 7BZ United Kingdom	
Telephone No.:	
Facsimile No.:	
Teleprinter No.:	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
WONG, Luet Lok University of Oxford Department of Chemistry South Parks Road Oxford OX1 3QR United Kingdom	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
BELL, Stephen Graham University of Oxford Department of Chemistry South Parks Road Oxford OX1 3QR United Kingdom	
State (that is, country) of nationality: GB	State (that is, country) of residence: GB
<input checked="" type="checkbox"/> Further applicants are indicated on a continuation sheet.	

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

CARMICHAEL, Angus Bishop
University of Oxford
Department of Chemistry
South Parks Road
Oxford OX1 3QR
United Kingdom

State (that is, country) of nationality:

GB

State (that is, country) of residence:

GB

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

State (that is, country) of nationality:

State (that is, country) of residence:

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

State (that is, country) of nationality:

State (that is, country) of residence:

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

State (that is, country) of nationality:

State (that is, country) of residence:

☐

Further applicants are indicated on another continuation sheet.

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCEThe following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ELLIS-JONES, Patrick George Armine
 J.A. KEMP & CO.,
 14 South Square,
 Gray's Inn,
 London, WC1R 5LX,
 United Kingdom.

Telephone No.:

+44 20 7405 3292

Facsimile No.:

+44 20 7242 8932

Teleprinter No.:

23676

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☐ as originally filed☐ as amended under Article 34the claims ☐ as originally filed☐ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☐ as originally filed☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English☒ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☐ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.**Box No. V ELECTION OF STATES**The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | |
|--|---|----------|
| 1. translation of international application | : | sheets |
| 2. amendments under Article 34 | : | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets |
| 5. letter | : | 1 sheets |
| 6. other (<i>specify</i>) | : | sheets |

For International Preliminary
Examining Authority use only

received not received

<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>

The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 4. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> separate signed power of attorney | 5. <input type="checkbox"/> nucleotide and or amino acid sequence listing in computer readable form |
| 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: | 6. <input type="checkbox"/> other (<i>specify</i>): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

ELLIS-JONES, Patrick George Armine

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.

☐ The applicant has been informed accordingly.

4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.

5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PATENT COOPERATION TREATY

PCT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ELLIS-JONES, Patrick-George-Armine
J.A. KEMP & CO. KEMP & CO
14 South Square
Gray's Inn
London WC1R 5LX
GRANDE BRETAGNE

REC'D 10 JUL 2000
SA
Action by.....

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing
(day/month/year)

0 6. 07. 00

Applicant's or agent's file reference
N.75121A PEJ

IMPORTANT NOTIFICATION

International application No.

PCT/GB 99/ 03873

International filing date (day/month/year)

19/11/1999

Priority date (day/month/year)

19/11/1998

Applicant

ISIS INNOVATION LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

15/06/2000

2. This date of receipt is:

- ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- ☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- ☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

- ☐ (If applicable) This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

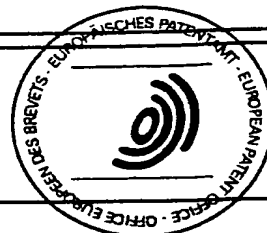


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D-80298 Munich
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Authorized officer

MORENO R A

Tel. (+49-89) 2399-2658



PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

ELLIS-JONES, Patrick George,
Armine
J.A. Kemp & Co
14 South Square
Gray's Inn
London WC1R 5LX
ROYAUME-UNI**J. A. KEMP & Co**

12 MAY 2000

Action by

Date of mailing (day/month/year) 28 April 2000 (28.04.00)	
Applicant's or agent's file reference N.75121A PEJ	IMPORTANT NOTIFICATION
International application No. PCT/GB99/03873	International filing date (day/month/year) 19 November 1999 (19.11.99)

1. The following indications appeared on record concerning:

☒ the applicant

 ☐ the inventor

 ☐ the agent

 ☐ the common representative

Name and Address

 ISIS INNOVATION LIMITED
 2 South Parks Road
 Oxford OX1 3UB
 United Kingdom

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person

 ☐ the name

 ☒ the address

 ☐ the nationality

 ☐ the residence

Name and Address

 ISIS INNOVATION LIMITED
 Ewert House
 Ewert Place
 Summertown
 Oxford OX2 7BZ
 United Kingdom

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input checked="" type="checkbox"/> the International Searching Authority	<input type="checkbox"/> the elected Offices concerned
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

 The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland

Authorized officer

S. Cruz



Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference N. 75121A PEJ	FOR FURTHER ACTION <small>see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</small>	
International application No. PCT/GB 99/ 03873	International filing date (day/month/year) 19/11/1999	(Earliest) Priority Date (day/month/year) 19/11/1998
Applicant ISIS INNOVATION LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.

/ GB 99/ 03873

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheets

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14, 17-20 all partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-14,17-20 all partially

A mutant P-450 cam in which amino acid 96 has been changed to an amino acid with a less polar side-chain. Also a nucleotide encoding it, a process for oxidising a terpene or a cycloalkene with it, a cell expressing it.

2. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which amino acid 87 has been changed.

3. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which amino acid 185 has been changed.

4. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which amino acid 247 has been changed.

5. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which an amino acid in the active site has been changed to an amino acid with a less polar side-chain, as far as not covered by (1)-(4).

6. Claims: 1-8,10,12-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 BM3 in which amino acid 47 has been changed.

7. Claims: 1-8,10,12-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 BM3 in which amino acid 51 has been changed.

8. Claims: 1-8,10,12-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 BM3 in which amino acid in the active site has been changed to an amino acid with a less polar side-chain, as far as not covered by (6)-(7).

9. Claims: 8-11,13,14,17-20 all partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A cell which expresses P-450 cam, or a fragment thereof or a naturally occurring homologue or a mutant thereof, and an electron transfer reductase and an electron transfer redoxin.

10. Claims: 8,10-14,17-20 all partially

A cell which expresses P-450 BM-3, or a fragment thereof or a naturally occurring homologue or a mutant thereof.

11. Claims: 14,17-20 all partially

A process for making a library of mutants of P-450 cam or P-450 BM3 by treating cells expressing P-450 cam or P-450 BM3 with a mutagen or when the cells are mutator cells, culturing them in conditions in which mutants are produced.

12. Claims: 15,16 all completely, 17,19,20 all partially

A process for selecting a mutant of P-450 cam or P-450 BM3 for its ability to oxidise a particular substance, comprising screening a group of mutants for their oxidation effect on the particular substance.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03873

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/53 C12N9/02 C12N1/21 C12N15/01 C12P7/02
/(C12N9/02,C12R1:40),(C12N9/02,C12R1:11),(C12N1/21,
C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 294 692 A (BRITISH GAS PLC)	1-6
Y	8 May 1996 (1996-05-08) abstract table 1 table 2A table 4 --- -/-	8-11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

28 February 2000

Date of mailing of the international search report

23.05.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03873

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ATKINS W M ET AL: "Molecular recognition in cytochrome P-450: alteration of regioselective alkane hydroxylation via protein engineering." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 111, 1989, pages 2715-2717, XP002131692 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 the whole document</p>	1-6
Y	<p>--- SIBBESEN O ET AL: "Putidaredoxin reductase-putidaredoxin-cytochrome P450cam triple fusion protein" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 271, no. 37, 13 September 1996 (1996-09-13), pages 22462-22469, XP002131693 AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD., US ISSN: 0021-9258 abstract</p>	8-11
A	<p>--- OLIVER C F ET AL: "A single mutation in cytochrome P450 BM3 changes substrate orientation in a catalytic intermediate and the regiospecificity of hydroxylation." BIOCHEMISTRY., vol. 36, 1997, pages 1567-1572, XP002131694 AMERICAN CHEMICAL SOCIETY. EASTON, PA., US ISSN: 0006-2960 abstract</p>	1-5
X	<p>--- ENGLAND P A: "The oxidation of naphthalene and pyrene by cytochrome P450cam" FEBS LETTERS., vol. 424, 13 March 1998 (1998-03-13), pages 271-274, XP002131695 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0014-5793 abstract</p> <p>--- -/--</p>	1-6

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03873

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ATKINS W M ET AL: "THE ROLES OF ACTIVE SITE HYDROGEN BONDING IN CYTOCHROME P-450 CAM AS REVEALED BY SITE-DIRECTED MUTAGENESIS" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 263, no. 35, 15 December 1988 (1988-12-15), pages 18842-18849, XP002025958 ISSN: 0021-9258 abstract -----</p>	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/03873

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2294692 A	08-05-1996	AU 705736 B	03-06-1999
		AU 3811795 A	31-05-1996
		CN 1171818 A	28-01-1998
		CZ 9701277 A	15-10-1997
		EP 0789770 A	20-08-1997
		WO 9614419 A	17-05-1996
		JP 10503658 T	07-04-1998
		NZ 294904 A	24-09-1998
		PL 319970 A	01-09-1997
		SK 54597 A	04-02-1998



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/53, 9/02, 1/21, 15/01, C12P 7/02 // (C12N 9/02, C12R 1:40) (C12N 9/02, C12R 1:11) (C12N 1/21, C12R 1:19)	A3	(11) International Publication Number: WO 00/31273 (43) International Publication Date: 2 June 2000 (02.06.00)
(21) International Application Number: PCT/GB99/03873 (22) International Filing Date: 19 November 1999 (19.11.99) (30) Priority Data: 9825421.2 19 November 1998 (19.11.98) GB (71) Applicant (for all designated States except US): ISIS INNO- VATION LIMITED [GB/GB]; Ewert House, Ewert Place, Summertown, Oxford OX2 7BZ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): WONG, Luet, Lok [GB/GB]; University of Oxford, Dept. of Chemistry, South Parks Road, Oxford OX1 3QR (GB). BELL, Stephen, Graham [GB/GB]; University of Oxford, Dept. of Chemistry, South Parks Road, Oxford OX1 3QR (GB). CARMICHAEL, Angus, Bishop [GB/GB]; University of Oxford, Dept. of Chemistry, South Parks Road, Oxford OX1 3QR (GB). (74) Agent: ELLIS-JONES, Patrick, George, Armine; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 17 August 2000 (17.08.00)
(54) Title: PROCESS FOR OXIDISING TERPENES (57) Abstract A process for oxidising a substrate which is an acyclic or cyclic terpene, or a cycloalkene; or a substituted derivative thereof, which process comprises oxidising said compound with a mutant haem-containing enzyme, the mutant comprising the substitution of an amino acid in the active site by an amino acid with a less polar side-chain. The enzyme is typically P450 _{cam} or P450 _{BM-3} . Cells and libraries of cells in which the process can be carried out or which can be used to select advantageous mutant enzymes are also provided.		

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INTERNATIONAL SEARCH REPORT

International Application No.

/GB 99/03873

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/53 C12N9/02 C12N1/21 C12N15/01 C12P7/02
 //(C12N9/02,C12R1:40),(C12N9/02,C12R1:11),(C12N1/21,
 C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 294 692 A (BRITISH GAS PLC) 8 May 1996 (1996-05-08)	1-6
Y	abstract table 1 table 2A table 4 --- -/-	8-11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search

28 February 2000

Date of mailing of the international search report

23.05.00

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No
 /GB 99/03873

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ATKINS W M ET AL: "Molecular recognition in cytochrome P-450: alteration of regioselective alkane hydroxylation via protein engineering." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 111, 1989, pages 2715-2717, XP002131692 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 the whole document</p>	1-6
Y	<p>--- SIBBESEN O ET AL: "Putidaredoxin reductase-putidaredoxin-cytochrome P450cam triple fusion protein" JOURNAL OF BIOLOGICAL CHEMISTRY., vol. 271, no. 37, 13 September 1996 (1996-09-13), pages 22462-22469, XP002131693 AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD., US ISSN: 0021-9258 abstract</p>	8-11
A	<p>--- OLIVER C F ET AL: "A single mutation in cytochrome P450 BM3 changes substrate orientation in a catalytic intermediate and the regiospecificity of hydroxylation." BIOCHEMISTRY., vol. 36, 1997, pages 1567-1572, XP002131694 AMERICAN CHEMICAL SOCIETY. EASTON, PA., US ISSN: 0006-2960 abstract</p>	1-5
X	<p>--- ENGLAND P A: "The oxidation of naphtalene and pyrene by cytochrome P450cam" FEBS LETTERS., vol. 424, 13 March 1998 (1998-03-13), pages 271-274, XP002131695 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0014-5793 abstract</p> <p>--- -/--</p>	1-6

INTERNATIONAL SEARCH REPORT

International Application No

GB 99/03873

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>ATKINS W M ET AL: "THE ROLES OF ACTIVE SITE HYDROGEN BONDING IN CYTOCHROME P-450 CAM AS REVEALED BY SITE-DIRECTED MUTAGENESIS" JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 263, no. 35, 15 December 1988 (1988-12-15), pages 18842-18849, XP002025958 ISSN: 0021-9258 abstract</p> <p>-----</p>	1-6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/ 03873

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheets

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14, 17-20 all partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-14,17-20 all partially

A mutant P-450 cam in which amino acid 96 has been changed to an amino acid with a less polar side-chain. Also a nucleotide encoding it, a process for oxidising a terpene or a cycloalkene with it, a cell expressing it.

2. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which amino acid 87 has been changed.

3. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which amino acid 185 has been changed.

4. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which amino acid 247 has been changed.

5. Claims: 1-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 cam in which an amino acid in the active site has been changed to an amino acid with a less polar side-chain, as far as not covered by (1)-(4).

6. Claims: 1-8,10,12-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 BM3 in which amino acid 47 has been changed.

7. Claims: 1-8,10,12-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 BM3 in which amino acid 51 has been changed.

8. Claims: 1-8,10,12-14,17-20 all partially

Same as invention 1, but pertaining to a mutant P-450 BM3 in which amino acid in the active site has been changed to an amino acid with a less polar side-chain, as far as not covered by (6)-(7).

9. Claims: 8-11,13,14,17-20 all partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A cell which expresses P-450 cam, or a fragment thereof or a naturally occurring homologue or a mutant thereof, and an electron transfer reductase and an electron transfer redoxin.

10. Claims: 8,10-14,17-20 all partially

A cell which expresses P-450 BM-3, or a fragment thereof or a naturally occurring homologue or a mutant thereof.

11. Claims: 14,17-20 all partially

A process for making a library of mutants of P-450 cam or P-450 BM3 by treating cells expressing P-450 cam or P-450 BM3 with a mutagen or when the cells are mutator cells, culturing them in conditions in which mutants are produced.

12. Claims: 15,16 all completely, 17,19,20 all partially

A process for selecting a mutant of P-450 cam or P-450 BM3 for its ability to oxidise a particular substance, comprising screening a group of mutants for their oxidation effect on the particular substance.

INTERNATIONAL SEARCH REPORT

on patent family members

International Application No

/GB 99/03873

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2294692 A	08-05-1996	AU 705736 B	03-06-1999
		AU 3811795 A	31-05-1996
		CN 1171818 A	28-01-1998
		CZ 9701277 A	15-10-1997
		EP 0789770 A	20-08-1997
		WO 9614419 A	17-05-1996
		JP 10503658 T	07-04-1998
		NZ 294904 A	24-09-1998
		PL 319970 A	01-09-1997
		SK 54597 A	04-02-1998
